Pharmacokinetics of chloramphenicol (CAP) in gilthead sea bream (Sparus aurata) and its in vitro activities against important bacterial fish pathogens

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Pharmacokinetics of chloramphenicol (CAP) in gilthead sea bream (Sparus aurata) and its in vitro activities against important bacterial fish pathogens

Tyrpenou A.E.,1 Rigos G.,2 Yiagnisi M.,2 Nengas I.,2 Alexis M.2

ABSTRACT. The pharmacokinetics of chloramphenicol (CAP) in gilthead sea bream (Sparus aurata), a warm water farmed fish species and its in vitro efficacy against important bacterial diseases of Mediterranean mariculture were investigated in this study. After an intravascular injection (10 mg/kg/fish), the distribution half-life (tl/2a) and the elimination half-life (tl/2ß) of CAP were calculated to be 1.6 and 69 h, respectively. Tissue penetration of CAP was found to be good since both the apparent volume of distribution of the drug at steady-state (Vss) and the apparent volume of the central compartment (V1) were calculated to be 1.13 and 0.90 L/kg. The total clearance (CLT) of the drug was slow (0.022 L/kg/h). The minimum inhibitory concentration (MIC) values of CAP in distilled water supplemented with 2% NaCl against Vibrio anguillarum serotype 1b, Photobacterium damsela subsp. piscicida, V. alginolyticus and V. fluvialis were determined to be 4.78 µg/mL, 55 µM Mg2+ and 19.13 µg/mL for V. alginolyticus and V. fluvialis, whereas showed no effect for V. anguillarum P. damsela subsp. Piscicida and V. damsela. The results indicate that CAP displayed a satisfactory kinetic profile and is eliminated fast from gilthead sea bream muscle; however, its high MIC values fast stress a possible inefficacy against important bacterial pathogens of Mediterranean mariculture.

Key words: pharmacokinetics, chloramphenicol, MIC, seabream
Η χλωραμφενικόλη (CAP) είναι ένα συνθετικά παράσηπος αντιβιοτικό ευρύ φάκα, το οποίο αποτελεί άμεσο χαρακτηριστικό για το Staphylococcus aureus. Την περίοδο του 1950, θαλασσινούς βακτηρίων στα ψάρια. Η χλωραμφενικόλη είναι παραδεμένη κατά τους βακτηριακούς προ-γραμματικούς ζωνών, συνδέοντας την υπομονέα 50S του βακτηριακού ριβοσώματος. Στο περιεχόμενο συστήμα, η χλωραμφενικόλη επικεντρώνεται κατά διαφόρων ασθενειών του ανθρώπου και των ζώων, αλλά λόγω προσβάσιμης της επικαιρότητας αναμνήσεων (IARC, 1990), καθώς και άλλων αναλύσεων του αιματοκύτταρου, η FDA/USA απερχόταν πλήρως τη χρήση της χλωραμφενικόλης (CAP) στα παραγωγικά ζώα (MVM, 1998). Σύμφωνα με το Ευρωπαϊκό Πρωτάθλημα Αντιμικροβιακής Αντιδράσης Εύρυχορών Προϊόντων (EMEA) η ιδιότητα της χρήσης της χωρίζεται και στην Ευρώπη, αλλά επειδή δεν υπάρχει αντιστοιχία στις ανεξαρτήτως διαδραστήρια, η χρήση της CAP επικεντρώνεται και στην Ευρώπη, αλλά επειδή δεν υπάρχει αντιστοιχία στις ανεξαρτήτως διαδραστήρια, η χρήση της CAP επικεντρώνεται κατά των παθογόνων βακτηρίων στα χτένια με 800 L με θαλασσινό νερό αλλιώς να κρασός (IARC, 1990). Νέες πληροφορίες για την ιδιοτήτα της υπομονέας 50S του Συμβουλίου (ΕΟΚ) 2377/90. Due to its effectiveness against bacterial pathogens, its illegal use is common (Robert et al. 1996). Limited information exists on the kinetics of CAP in farmed aquatic organisms mainly confined to fresh water fish (Skare et al. 1974, Cravedi et al. 1985) and prawns (Liu and Liu, 1993). Some data on the minimum inhibitory concentrations (MIC) of CAP against bacteria pathogens of scallop (Pecten maximus) were recently published (Torkildsen et al. 2000). The aim of the present study was to provide information on the pharmacokinetic properties of CAP in gilthead sea bream (Sparus aurata), which is the leading warm water farmed species in Mediterranean area and to determine MIC values against the most important bacterial pathogens affecting this industry, including an investigation of the effect of seawater cations to the antimicrobial activity of CAP.

MATERIALS AND METHODS

Chloramphenicol (CAP) was obtained from Sigma Chemical Co (St. Louis, USA). The chemicals used for processing the samples (HPLC-grade) were obtained from Labscan Ltd (Dublin, Ireland), unless otherwise stated. Fishes were anesthetized with quinaldine (Sigma Chemical Co, St. Louis, USA).

Experimental design
Fifty gilthead sea bream averaging 300±30 g were acclimatized for two weeks prior to initiation of the study. Fishes were maintained in cylindrical fiberglass tanks (800 L) receiving 36% sea water. Photoperiod was kept at 12 h dark - 12 h light and water temperature was 19±0.5 °C. Fishes were starved for two days before the experiment and remained unfed during the experiment.

Drug administration
Prior to injection fishes were anesthetized with quinaldine (2 mL/L) dissolved in salt water and were weighed. Fishes received 100 μL of medicated solution including the dose (10 mg/kg fish) in sterile saline. To ensure the correct position of the needle (caudal vein)
βομβιστών με φωτοπερίοδο η οποία διατηρήθηκε σε 12 h οικοτάδι και 12 h φως και με μέση θερμοκρασία του νερού 19±0,5°C. Πριν από την έναρξη του πειράματος τα γάρια παρέμειναν νηπιαία για δύο μέρες και χωρίς τροφή κατά τη διάρκεια του πειραματισμού.

Χορήγηση του φαρμάκου

Πριν από την ενδοφλεβική χορήγηση της CAP τα γάρια αναιμώνθηκαν σε χρονική στιγμή προηγουμένως ανεκπεμφόντας με 2 mL/L θαλάσσινο νερό και ζυγίζοντας. Στη συνέχεια εγκατέστησαν ενδοφλεβικά 100 mL από το φαρμακευτικό διάλυμα σε αποστειρωμένο φυσιολογικό νερό στη δόση των 10 mg/kg βιομάζας. Για να βεβαιωθούμε για την ακρίβη θέση της βελόνας στην ουραία φλέβα (Microlance 23G 1,6 x 30, Becton Dickinson SA, Fraga, Spain), πριν και μετά από την ένταξη γενόταν αναφόρηση αίματος. Τα γάρια, τα οποία παρουσίαζαν έντονη αιμορροϊκή εκτροπία, αποτίθενταν από τον πειραματισμό και αντικαθίσταντο από άλλα. Μετά από την ενδοφλεβική χορήγηση του φαρμάκου λαμβανόταν διάρκεια αίματος 1 mL από την ουραία φλέβα κάθε φωτιά από τα 8 ψάρια κάθε δείγματος για χρονική στιγμή δειγματοληψίας, δηλαδή ανά 1, 2, 4, 8, 16, 32, 64 και 128 h. Μετά τη συλλογή των δειγμάτων αίματος τα γάρια θανατώνταν με κόψιμο της νωτιαιας χορδής των 10 mg/kg βιομάζας. Για να βεβαιωθούμε για την ακρίβεια του πειραματισμού.

Υγροχρωματογραφική ανάλυση

Η μεθόδος η οποία εφαρμόσθηκε για τον προσδιορισμό της CAP στα δείγματα της σάρκας προηγουμένως αναιμώνθηκαν την έτερη μεθόδος με μικρές προσποιήσεις: Συνηφόρησαν, 0,2 mL πλάσμα αίματος εγκατέστησαν με 2 x 2 mL οξίνο σιλικονίου εξαπλεθέασαν με αναφόρηση στο νεότερο και στις πετράδες, συσκευασία και εκσυμφερόταν σε 3500 x g για 10 min στους 10°C και την αναφόρηση της βελόνας στην ουραία φλέβα (Microlance 23G18,0-6 x 30, Becton Dickinson SA, Fraga, Spain) από το φαρμάκο. Τα δείγματα εκχυλίζονταν μέχρι την ανάλυση.

Drug Analysis

The methodology for the analysis of CAP in muscle samples followed that of Tyrenou et al. (2002). For analysis of CAP in plasma, minor modifications were made. Briefly, 0.2 mL plasma were extracted with 2 mL ethyl acetate by vortex mixer and sonication, centrifuged at 3500 x g for 10 min at 10°C and the supernatant was transferred to another glass vial. The sample was extracted once more with 2 mL ethyl acetate and the combined extracts were evaporated under a gentle stream of nitrogen at 55°C. The remaining oily residue was dissolved in 1 mL of 3% NaCl and vortexed and sonicated. A partition washing step with 3 mL n-pentane was followed and after centrifugation the upper layer was rejected by aspiration. CAP was back extracted with 2 x 2 mL ethyl acetate and the combined extracts were evaporated to dryness with nitrogen at 55°C. The vial was rinsed with 1 mL n-hexane and rinsing was poured into the mini column (Sep-Pak Light Silica Cartridges, Waters MA, USA). Rinsing of the mini column for the removal of the undesired matrix compounds was made with 2 mL n-hexane and selective desorbing of CAP from silica gel was made with 1 mL of HPLC methanol. The eluate was evaporated to dryness under a gentle stream of nitrogen at 55°C and the residue was redissolved in 0.5 mL of the mobile phase (methanol: HPLC-grade water, 30:70[v/v]), filtered through 0.22 μm filter into 1 mL the autosampler’s glass vial. An aliquot of 100 μL was immediately injected into a Photo Diode Array detector (Waters MA, USA) monitored at λ = 278 nm. Analytes were separated using a Zorbax SB-C18 (5 μm) column (25 cm x 4.6 mm i.d.) (Waters MA, USA) maintained at a temperature of 50°C. Mean recovery achieved was 88.62±9.65 % for a range of 10, 25, 50, 100, 200 μg/kg blank fortified samples (n = 4). The limit of detection (LOD) and the limit of quantification (LOQ) were 5 and 10 μg/kg, respectively. Control of the system, data acquisition and peak integration were performed by the software Millennium2® Workstation plus Millennium2® System Suitability software Version 3.05.01. CAP concentrations were extrapolated from the standard curves.

Pharmacokinetic analysis

The data were analyzed for the best fit to a two or three-compartment open pharmacokinetic model, using non-

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υγροχρωματογράφο για ανίχνευση της CAP από τον ανιχνευτή P.Η. Reader (ASYS Hitech, Austria). MIC testing was carried out with the broth microdilution method at 22°C. Antibiotic solution was made by dissolving CAP in distilled water. Dilutions were made in Muller Hinton broth (MHB) dissolved in either distilled water supplemented with 2% NaCl or additionally including 10 mM Ca²⁺ and 55 mM Mg²⁺ (which approximates the content of the Mediterranean salinity), since previous studies have stressed the impact of these cations on the activity of drugs in marine environment (Barnes et al., 1995, Pursell et al. 1995, Lunestad and Samuelsen, 2001). CAP concentrations were evaluated in the test wells ranged from 0.019 to 38.25 μg/mL.

All bacterial pathogens which have been used in this study were derived from diseased farmed gilthead sea bream and sea bass from 2000 to 2001 and were received from the Fish Pathology Laboratory of the National Centre for Marine Research (NCMR), where they were kept at -70°C in 20% glycerin. The bacteria inflict the most significant losses in euryhaline mariculture and include the primary pathogens Vibrion anguillarum serotype 1b, Photobacterium damselae subsp. piscicida and V. damsela and the opportunistic bacteria V. alginolyticus and V. fluvialis. Only one representative strain of each bacterial pathogen in Greece was tested. The Vibrio species and P. damselae subsp. piscicida were identified according to Mercedes and Blanch (1994) and Bakopoulos et al. (1995), respectively. Bacterial aliquots were taken from glycerol stocks and maintained on Tryptic Soya Agar (TSA) supplemented with 2% NaCl and 55 mM Mg²⁺ (which approximates the content of the Mediterranean salinity), since previous studies have stressed the impact of these cations on the activity of drugs in marine environment (Barnes et al., 1995, Pursell et al. 1995, Lunestad and Samuelsen, 2001). MIC determinations

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linear regression analysis programs (NLREG 2001, P.H. Sherrod), following a semi-logarithmic plot of the data and least square fitting (Ritschel, 1986). Diffusion processes were all assumed to follow first order kinetics. Calculations of the apparent volume of distribution at steady state (Vss), the mean residence time (MRT) and the total body clearance (CLt) were performed in a model independent way. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule (Ritschel, 1986). The elimination half-life were calculated from the equation t₁/₂ = ln2/β (Baggot, 1977).

MIC determinations

MICs were carried out with the broth microdilution method at 22°C. Antibiotic solution was made by dissolving CAP in distilled water. Dilutions were made in Muller Hinton broth (MHB) dissolved in either distilled water supplemented with 2% NaCl or additionally including 10 mM Ca²⁺ and 55 mM Mg²⁺ (which approximates the content of the Mediterranean salinity), since previous studies have stressed the impact of these cations on the activity of drugs in marine environment (Barnes et al., 1995, Pursell et al. 1995, Lunestad and Samuelsen, 2001). CAP concentrations were evaluated in the test wells ranged from 0.019 to 38.25 μg/mL.

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piscicida, V. damsel, και τα ευκαιριακά βακτήρια V. fluvialis και V. damsela. Από κάθε παθογόνο βακτήριο εξετάσθηκε ένα μόνο σπέρμα του οποίου είναι αντι- προσωπικιστικό στην Ελλάδα. Τα είδη Vibrio και Photobacterium damsela subsp. piscicida, V. damsela και τα ευκαιριακά βακτήρια V. fluvialis, V. damsela, V. fluvialis και V. alginolyticus, υπολογίσθηκαν σε 1,13 του κεντρικού διαμερίσματος (V των μονοεκθετικών καμπύλων).

ΑΠΟΤΕΛΕΣΜΑΤΑ

Αποτελέσματα συμφώνησαν εξίσου με τα δύο μονοεκθετικά κεντρικά διαμερίσματα. Οι συγκέντρωσεις της CAP στο πλάσμα του αίματος στο χρόνο t = 0, το σημείο ωθήσεως της CAP, αποτελούν την επίγεια σημείωση (ND) και η οποία μέσος ύψος (με την επίγεια σημείωση ωθήσεως). Οι συγκέντρωσεις της CAP στο μυϊκό ιστό είναι 1.99 ± 0.18 με επίπεδα της CAP που βρέθηκαν να είναι 0,2 στα 480 nm). Τα επίπεδα της CAP που βρέθηκαν στο μυϊκό ιστό του κεντρικού διαμερίσματος (V των μονοεκθετικών καμπύλων) ανήκε σε 1,13 του κεντρικού διαμερίσματος (V των μονοεκθετικών καμπύλων). Οι συγκέντρωσεις της CAP στο μυϊκό ιστό είναι 1.99 ± 0.18 με επίπεδα της CAP που βρέθηκαν να είναι 0,2 στα 480 nm).

AUC: Ολική σωματική απομάκρυνση
da 1/2α: Μέσος χρόνος παραμονής
d Vc (ss): Apparent volume of distribution of the drug at steady-state
d CLd: Ολική σωματική απομάκρυνση
d MRT: Mean residence time

Πίνακας 2. Παράμετροι φαρμακοκινητικής της CAP στο πλάσμα του αίματος (μικρά ιατρικά) και στο μυϊκό ιστό (μικρά ιατρικά) (mean ± SD, n = 3-5) μετά από μία ενδοφλέβια χορήγηση (10 mg/kg/ fish) στην τσιπούρα στους 19°C.

<table>
<thead>
<tr>
<th>Χρόνος στιγμές (όρες μετά τη χορήγηση)</th>
<th>Παράμετρος</th>
<th>Πλάσμα αίματος</th>
<th>Μυϊκός ιστός</th>
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<tr>
<td>1</td>
<td>t1/2a (h)</td>
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<td>69.30</td>
</tr>
<tr>
<td>2</td>
<td>t1/2b (h)</td>
<td>69.30</td>
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<tr>
<td>3</td>
<td>Vc (L/kg)</td>
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</tr>
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<td>Vd (L/kg)</td>
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</tr>
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<td>5</td>
<td>AUC (μg/mL)</td>
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<td>152</td>
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<tr>
<td>6</td>
<td>CLd L/kg/h</td>
<td>0.02</td>
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</tr>
<tr>
<td>7</td>
<td>MRT (h)</td>
<td>51.75</td>
<td>51.75</td>
</tr>
</tbody>
</table>

ND : not detected
Α. Ε. ΤΥΡΠΕΝΟΥ, Γ. ΡΗΓΟΣ, Μ. ΓΙΑΓΝΙΣΗ, Ι. ΝΕΓΚΑΣ, Μ. ΑΛΕΞΗ

καμπύλη απομάκρυνσης της χλωραμφενικόλης - CAP από το μυϊκό ιστό
chloramphenicol - CAP elimination curve from muscle tissue
καμπύλη απομάκρυνσης της χλωραμφενικόλης - CAP από το πλάσμα
chloramphenicol - CAP elimination curve from blood plasma

Εικόνα 1. Καμπύλες απομάκρυνσης της χλωραμφενικόλης από το μυϊκό ιστό και το πλάσμα του αίματος μετά από ενδοφλέβια χορήγηση.
Figure 1. Chloramphenicol elimination curves from muscle tissue and blood plasma after intravascular administration to gilthead seabream Sparus aurata.

ΣΥΖΗΤΗΣΗ
Η παρούσα μελέτη μας πληροφορεί για τις φαρμακοκινητικές ιδιότητες της CAP στο κυριότερο είδος της Μεσογειακής ιχθυοκαλλιέργειας, την τσίπουρα, και για τις τιμές MIC ενάντια των πλέον σημαντικών παθογόνων βακτηρίων, τα οποία εμπλέκονται στη δραστηριότητα αυτή. Στη βιβλιογραφία δεν αναφέρονται συγκριτικές φαρμακοκινητικές μελέτες σε τσίπουρα.

RESULTS
The data conformed equally to a two and three-compartment model. Since the three-compartment model gave α = β, the plasma concentration versus time curve was preferably calculated from the two-exponential equation:

\[ C = Ae^{-\alpha t} + Be^{-\beta t} \]

where C is the plasma concentration, t is the time, α and β are the slopes of mono-exponential declining curves and A and B are the zero time plasma concentrations. The calculated pharmacokinetic parameters of CAP in gilthead sea bream are presented in Table 1. Following the injection, the distribution half-life (t_{1/2a}) and the elimination half-life (t_{1/2ß}) of CAP from plasma were found to be 1.6 and 69 h, respectively. The apparent volume of distribution of the drug at steady-state (Vd(ss)) and the apparent volume of the central compartment (Vc) were estimated to be 1.13 and 0.90 L/kg, respectively. The total clearance of the drug (CLT) was found to be 0.022 L/kg/h and the mean residence time (MRT) was 51 h. The plasma and muscle CAP levels obtained are shown in Table 2. Maximum values were observed for muscle at 2 h following injection, with levels declining in subsequent samplings. CAP became undetectable from muscle at 64 h post administration.
Το Παθογόνο βακτηρία

API20E Κωδ. αριθμός API20E Code number

<table>
<thead>
<tr>
<th>Παθογενής Βακτηρία</th>
<th>MIC 2% NaCl</th>
<th>MIC + κατιόντα</th>
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<td>Vibrio anguillarum lb</td>
<td>4.78</td>
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<td></td>
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<tr>
<td>Photobacterium damsela subsp. Piscicida</td>
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<tr>
<td>Vibrio alginitolyticus</td>
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<td>Vibrio damselae</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio fluvialis</td>
<td>4.78</td>
<td>19.13</td>
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</tr>
</tbody>
</table>

**DISCUSSION**

The present study provides information on the pharmacokinetics of CAP in the leading species of Mediterranean fish farming, the gilthead sea bream and reveal MIC values against important bacterial pathogens involved in this industry. No comparable studies on the kinetic profile of CAP in other farmed fishes exist. However, some comparisons can be made by integrating the pharmacokinetics of three common and registered antibacterial agents in several European countries, including oxytetracycline (OTC), oxolinic acid (OA) and flumequine (FLU) in the same fish species and at similar water temperatures (Rigos et al. 2002, Rigos et al. 2003a, Rigos et al. 2003b).

The distribution half-life (t½α) of CAP (1.6 h) is longer compared to the values calculated for OTC, OA and FLU in gilthead sea bream (0.2 – 1.5 h) (Rigos et al. 2002, Rigos et al. 2003a, Rigos et al. 2003b), indicating that CAP is distributed slowly to the tissues of gilthead sea bream from the blood compartment. Similarly, the elimination half-lives (t½β) of OTC, OA and FLU (12 - 50 h) (Rigos et al. 2002, Rigos et al. 2003a, Rigos et al. 2003b) are shorter compared to the values estimated in the aforementioned studies for FLU, OTC and OA (0.05 - 0.15 L/kg/h). The apparent volume of distribution of CAP at steady-state (Vdss) (1.13 L/kg) is higher compared to that calculated for FLU (0.57 L/kg) is higher compared to that calculated for FLU (0.57 L/kg).
The MIC values of the drug, using distilled water supplemented with 2% NaCl, were found to be below 1 µg/mL only for V. damsela, whereas corresponding values against V. anguillarum serotype lb, Photobacterium damsela subsp. piscicida, V. alginolyticus and V. fluvialis were much higher (4.78 µg/mL). The addition of marine cations in the medium resulted to a 4-fold reduction in CAP activity against P. damsela, V. anguillarum, P. damsela subsp. piscicida, V. alginolyticus and V. fluvialis, whereas showed no effect for V. damsela subsp. piscicida, V. alginolyticus and V. fluvialis.

The CAP targets the bacterial ribosome, differences on the reduction of the activity of the drug against the bacteria were observed in the presence of seawater components to the activity of CAP along with other exogenous compounds (Stegeman, 1989). Additionally, the elimination of CAP from gilthead sea bream muscle is shorter compared to that found in rainbow trout (Onchorhyncus mykiss) (Skare et al. 1974), where CAP residues were measured even after 8 days post administration, when the drug was given in gelatin capsules (50 mg/kg fish). In general, the processing and elimination of drugs is faster in marine compared to fresh water species (Ishida, 1992). A rapid CAP elimination from giant tiger prawn (Peneaus monodon) and kuruma prawn (P. japonicus) muscle and serum has been demonstrated in the study of Liu and Liu (1993), where the drug was administered orally (50 mg/kg prawn) or by 24 h-bath (80 µg/L) treatment and become undetectable within 24 h.

In Figure 1 we can see CAP elimination from blood plasma and muscle tissue, the mean concentrations found and the standard deviations calculated.

The MIC values of CAP (Rigos et al., 2003a), but smaller to the values found for OTC and OA (2.11 – 2.90 L/kg) (Rigos et al. 2002, Rigos et al., 2003b). The V_diss value of CAP indicates an adequate distribution of the drug into tissues which is of vital importance against pathogenic bacteria localized in poorly vascularized tissues.

It has been reported that CAP is disappeared much faster compared to OTC from the muscle of fresh water fishes (Anhalt, 1977). This is in agreement with residue comparisons (OTC vs CAP) that can be made from OTC studies in gilthead sea bream (Rigos et al., 2003b). In the present study, CAP was undetectable in gilthead sea bream muscle at 64 h post-injection, whereas OTC in the above unpublished work was measured at even 7.5 days post-injection. Since fish species and water temperature are identical between these studies, this is probably due to the differences in the detoxification process between CAP and OTC mainly directed to microsomal cytochrome P450-dependent mixed-function oxidase system, which is the primary mechanism to catalyse oxidative metabolism of a variety of drugs along with other exogenous compounds (Stegeman, 1989). Additionally, the elimination of CAP from gilthead sea bream muscle is shorter compared to that found in rainbow trout (Onchorhyncus mykiss) (Skare et al. 1974), where CAP residues were measured even after 8 days post administration, when the drug was given in gelatin capsules (50 mg/kg fish). In general, the processing and elimination of drugs is faster in marine compared to fresh water species (Ishida, 1992). A rapid CAP elimination from giant tiger prawn (Peneaus monodon) and kuruma prawn (P. japonicus) muscle and serum has been demonstrated in the study of Liu and Liu (1993), where the drug was administered orally (50 mg/kg prawn) or by 24 h-bath (80 µg/L) treatment and become undetectable within 24 h.

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318 Α. Ε. ΤΥΡΠΕΝΟΥ, Γ. ΡΗΓΟΣ, Μ. ΓΙΑΓΝΙΣΗ, Ι. ΝΕΓΚΑΣ, Μ. ΑΛΕΞΗ
Το οροσύμπος lb, το Photobacterium damsela subsp. piscicida, το V. anguillarum serotype lb, το Photobacterium damsela subsp. piscicida, το VaUghiLLoatuς και το V. flaUial. Για να επεβεβαιωθεί αυτή η υπόθεση απαιτούνται περισσότερα πειράματα τυχαίας μόλυνσης (challenge) με αυτά τα παθογόνα βακτήρια ακολουθούμενα από θεραπεία με CAP.

Συμπερασματικά, η CAP παρουσιάζει ικανοποιητική φαινομενολογία συμπερασμάτων με μια χρήση απομέιωσης από το μετάνιο άλλων τοξικότοξων. Όμως, οι βιολογικές τιμές MIC σε συνδυασμό με τις χαμηλές συγκέντρωσες της CAP στο πλάσμα του αίματος μετά από ενδοδομή χρήση, συνδυάζονται με μια πιθανότητα αποτελεσμάτων με εμπλοκή στην φαινομενολογία. Μια χορήγηση CAP από το στόμα προφανώς θα είχε δείξει τη θογόνα βακτήρια ακολουθούμενα από θεραπεία με CAP.

Αφεθείτε να υπογραμμιστεί ότι η CAP δεν θα έπρεπε να θεωρείται ως αποτελεσματική ως αποτέλεσμα του βίαιου σιδηραματικού χαμηλού επίπεδου στο πλάσμα του αίματος. Μια χορήγηση CAP από το στόμα προφανώς θα είχε δείξει τη θογόνα βακτήρια ακολουθούμενα από θεραπεία με CAP.

Ishida N (1992) Tissue levels of oxolinic acid after oral or intravascular administration in freshwater and seawater rainbow trout. Aquaculture, 102:9-15

BIBLIOGRAPHIA - REFERENCES

Ishida N (1992) Tissue levels of oxolinic acid after oral or intravascular administration in freshwater and seawater rainbow trout. Aquaculture, 102:9-15


Skare JU, Hastein T, Froelie A (1974) Determination of residues of chloramphenicol in rainbow trout (Salmo gairdneri) and Atlantic salmon (Salmo salar). Nord Veterinary Medicine, 26:108-115


